ACTIVITY OF THE HSP90 INHIBITOR, AT13387, IN ALK-DRIVEN TUMOR MODELS.

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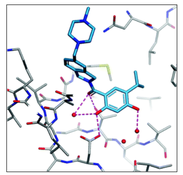
INTRODUCTION

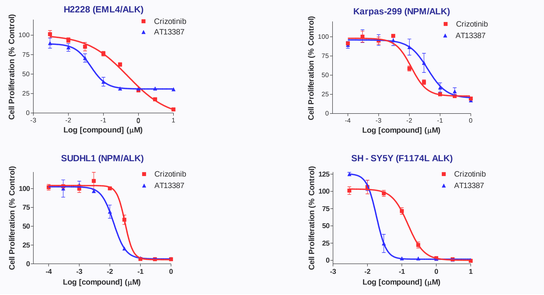
Anaplastic lymphoma kinase (ALK) is activated in several different cancers through translocation, mutation or amplification making ALK an important cancer target in a number of indications. ALK fusions have been identified in both non-small cell lung cancer (NSCLC) (EML4/ALK) and anaplastic large cell lymphoma (ALCL) (NPM/ALK), whilst point mutations in ALK activate the kinase in neuroblastoma (F1174L). The ALK inhibitor, crizotinib, has been successful in the treatment of ALK-positive NSCLC, but resistance to this inhibitor arises rapidly. A number of diverse resistance mechanisms to crizotinib have been identified including point mutations in the ALK protein itself and activation of alternative signalling pathways.

The ALK fusion proteins are sensitive clients for HSP90, as are several of the proteins involved in ALK signalling, making inhibition of HSP90 another potential approach to treating ALK-driven tumors and potentially overcoming the multiple mechanisms of crizotinib resistance. AT13387 is a potent (K_d 0.71 nM) fragment-derived HSP90 inhibitor currently being tested in clinical trials. Here we show that AT13387 is effective in a number of *in vitro* and *in vivo* ALK-dependent tumor models driven by different forms of activated ALK.



AT13387 inhibits the proliferation of ALK-dependent cell lines

Cell line	ALK Status	Crizotinib Inhibition of Proliferation IC_{50} (nM)	AT13387 Inhibition of Proliferation IC_{50} (nM)
H2228	EML4/ALK (NSCLC)	750	60
Karpas-299	NPM/ALK (Lymphoma)	15	38
SUDHL1	NPM/ALK (Lymphoma)	25	12
SH-SY5Y	F1174L ALK (Neuroblastoma)	150	19

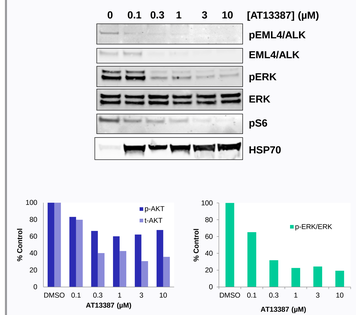


AT13387 potently inhibited proliferation of a number of cell lines driven by different forms of activated ALK (fusions and point mutations) and with different sensitivities to crizotinib.

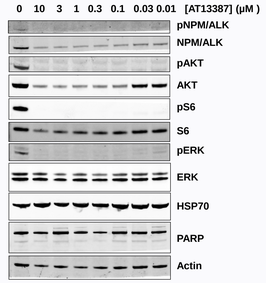
AT13387 IS EFFECTIVE AGAINST CELL LINES DRIVEN BY ALK

AT13387-treament depletes client proteins and inhibits signalling in H2228 and Karpas-299 ALK dependent cell-lines

H2228 (EML4/ALK)

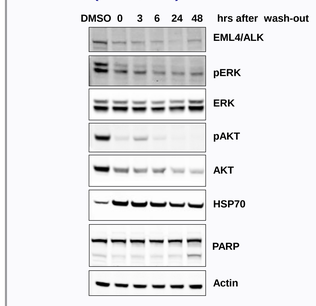


KARPAS-299 (NPM/ALK)

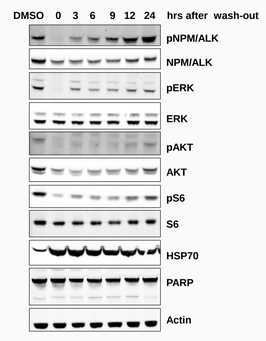


Cells were treated with the indicated concentrations of AT13387 for 24 h. Samples were then taken for analysis. AKT, phospho-AKT and phospho-ERK levels were measured by Mesoscale Discovery (MSD).

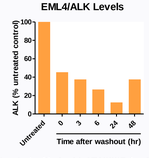
H2228 (EML4/ALK)



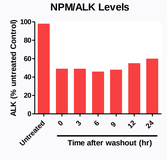
KARPAS-299 (NPM/ALK)



EML4/ALK Levels



NPM/ALK Levels

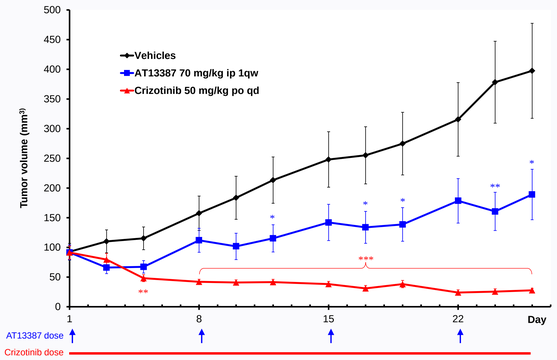


Cells were treated with 1 μM AT13387 for 16 h, washed and reincubated with fresh culture medium. Samples were taken at the indicated times post wash for analysis. ALK levels were quantified by scanning western blots using the Odyssey infrared imaging system (Licor).

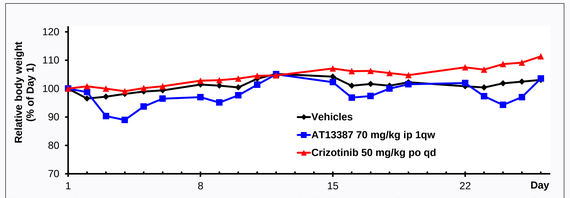
AT13387 treatment led to the depletion of both EML4/ALK and NPM/ALK fusions. Signalling through the AKT and ERK pathways was also inhibited as demonstrated by the reduction in levels of pAKT and pERK.

AT13387 INHIBITS TUMOR GROWTH AND SIGNALING IN ALK-DEPENDENT XENOGRAFT MODELS

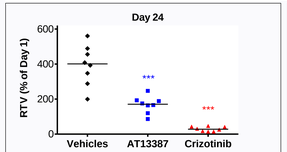
AT13387-treatment inhibits tumor growth in an ALK-dependent H2228 (EML4/ALK) xenograft model



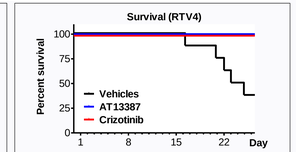
SCID mice bearing subcutaneous H2228 tumors were treated with 70 mg/kg of AT13387 once a week, 50 mg/kg of crizotinib once daily or vehicles. AT13387 was dissolved in 17.5% (2-hydroxypropyl)-β-cyclodextrin and administered intraperitoneally. Crizotinib was suspended in water and administered orally. Data represent mean SEM (N=8). * p<0.05; ** p<0.01; *** p<0.001 vs vehicle control.



Change in body weight of the tumor-bearing animals treated with AT13387 or crizotinib. Each data point is the median of the group.

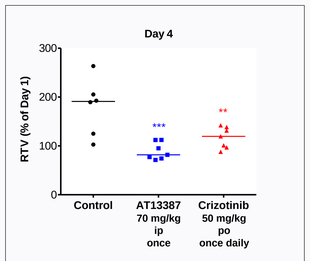


Analysis of the tumors on Day 24 when the greatest antitumor effect was observed. Data represent individual tumors and median. *** p<0.001 vs control.

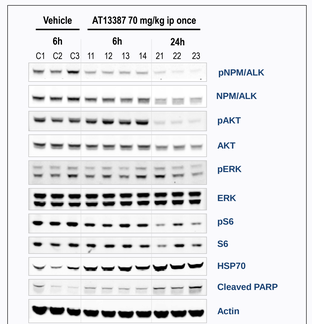


Survival analysis using RTV4 as the endpoint. Median survival of control mice was 24 days. None of the drug-treated tumors reached RTV by Day 26.

Tumor growth inhibition, client knockdown and inhibition of signalling in Karpas-299 tumor xenograft bearing mice treated with AT13387



SCID mice bearing subcutaneous Karpas-299 tumors were treated with 70 mg/kg of AT13387, 50 mg/kg of crizotinib or vehicles using the same schedule as the H2228 study.
Data represent individual tumor volumes (relative to Day 1 of treatment) and median. ** p<0.01; *** p<0.001 vs control.



Karpas-299 tumor-bearing mice were treated with 70 mg/kg of AT13387 and tumors analysed at the indicated time points.

AT13387 treatment inhibits tumor growth driven by both the EML4/ALK (H2228) and NPM/ALK (Karpas-299) fusions. ALK is also depleted and signalling inhibited in ALK-dependent tumor xenografts.

SUMMARY AND CONCLUSIONS

- The HSP90 inhibitor AT13387 is effective in ALK-driven tumor models regardless of the activated form of ALK.
- Treatment with AT13387 depletes both the EML4 and NPM-fused forms of ALK, which are both HSP90 clients, and inhibits signalling through both the ERK and AKT pathways.
- *In vivo*, AT13387 inhibits tumor growth in models driven by both ALK-fusion proteins.
- AT13387 is currently being tested clinically in Phase II trials for GIST, NSCLC and prostate cancer.
- Resistance to the ALK inhibitor, crizotinib, arises rapidly in the clinic. These data suggest that treatment with AT13387 could be a potential approach for treating ALK-driven tumors including those resistant to crizotinib.

References

Woodhead et al (2010) J Med Chem 53, 5956-69
Graham et al (2012) Cancer Sci 103, 522-7
Smyth et al (2012) Mol Cancer Ther 11, 1799-1808

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Poster can be downloaded from www.astx.com